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Background
Bacillus subtilis has been considered a model organism to study formation of complex multicellular structures called biofilm. Impaired respiration is one of the environmental signals triggering biofilm formation in *B. subtilis*. *Vitreoscilla* hemoglobin (VHb) is known to supply oxygen to respiratory chain and hence improves aerobic growth of variety of bacteria including *Bacillus* spp. Although VHb improves respiration, very little efforts have been made in elucidating its effect on biofilm formation. Here, we report the effect of incorporation of VHb on biofilm formation in *B. subtilis* DK1042 in different conditions.

Methods
B. subtilis DK1042 was genetically modified to develop two Integrants NRM1113 and NRM1114 containing *vgb-gfp* operon under 2 and 5 copies of P43 promoters, respectively, at *amyE* locus by double cross over events. Promoter strength was characterized by measuring the GFP fluorescence of both the integrants. Effect of VHb on biofilm formation by integrants and wildtype (WT) was assessed on both solid and pellicle biofilm in lysogeny broth (LB) and LB supplemented with 1% glyceol and 0.1 mM manganese (LBGM). Pellicle biofilms were assayed by crystal violet staining method. Solid biofilms were monitored for the development of complex architecture. Sporulation efficiency was determined by plate count method. Effect of VHb was also monitored by exposing the cells to 6 % salt stress in solid LB medium.

Results
Time dependent analysis of cell growth and fluorescence of *B. subtilis* integrants revealed that integrant NRM1114 having higher copies of P43 promoter upstream of *vgb-gfp* operon showed two fold increase in fluorescence/OD_{600} as compared to NRM1113 in both minimal medium and Luria Bertani medium. Moreover, production of brown pigment upon sporulation was reduced in integrants as compared to WT in minimal medium. Biofilm formation was significantly reduced on LB and LBGM in integrants as compared to WT. Integration of *vgb* also decreased biofilm-associated sporulation in both the integrants as compared to WT on LB and LBGM agar as well as LB agar supplemented with 6% NaCl.

Conclusion
Biofilm formation is an adaptation by microorganisms to withstand variety of environmental insults. Reduced biofilm formation and sporulation by both the integrants harboring *vgb* suggests better capacity to survive adverse environment. VHb is known to improve antioxidant status of the host cells. Thus, improved aerobic metabolism and detoxification of ROS by constitutive

expression of VHb together contribute to maintaining the vegetative state longer than the WT. Incorporation of VHb in other *Bacillus* spp. may have similar beneficial effects during their sessile lifestyle in rhizosphere that may enhance their performance as Plant Growth Promoting Rhizobacteria (PGPR). It would be interesting to understand the molecular processes involved in VHb modulated biofilm formation.